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EVALUATION OF WATER SANITATION OPTIONS FOR POULTRY PRODUCTION

Evaluation of Water Sanitation Options for Poultry Production

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Poultry Science

by

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Tribhuvan University
Bachelors in Veterinary Science and Animal Husbandry, 2010

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ABSTRACT

An evaluation of poultry farm water supplies was conducted to determine the value and impact of water system sanitation practices in commercial broiler houses on microbial levels. Water line cleaning between flocks using concentrated disinfectant solution before placing chicks reduced biofilms retained in the lines to a safe level. Occasional microbial surges were noticed during different points of flock grow-out period even when daily water sanitation was present indicating water is highly susceptible to microbial contamination. However, the daily water sanitation practice controlled the occasional microbial surges in water from sustaining and kept drinking water to a microbiologically acceptable level. Regardless of the line cleaning between flocks and daily water sanitation practice, biofilm buildup in water lines reoccurred by the 6th week of bird grow-out period requiring a mandatory line cleaning between flocks to optimize system hygiene and to ensure microbiologically safe water for the next flock of chicks.

The second study involved using hydrogen peroxide as an alternative disinfectant to chlorine for water sanitation. An in vitro trial was conducted to evaluate commercially available hydrogen peroxide products at their recommended concentrations for residuals and efficacy over time. Effective Residual Concentration (ERC) of 25-50 ppm of hydrogen peroxide in test solution (drinking rate for birds) started in the lowest concentration tested at 59. 14 ml of product added to 3780 ml of water creating stock solution for all products tested. At this concentration, all products maintained the ERC level at least for 3 days of preparing test solutions, with tendency of holding this residual level for a longer period by stabilized products than non-stabilized. Significant bacterial reductions within an hour of contact time were achieved in 48

hours post treatment microbial water introduction in test solutions as challenge. However, higher residuals or longer contact time was required for mold control.

Key words: water sanitation, microbial levels, disinfectants, efficacy

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Pramir Maharjan

DEDICATION

I devote my thesis work to my mother Lila Maharjan
and my father Maththa Bahadur Maharjan.

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INTRODUCTION

Maintaining drinking water quality for poultry is an important nutritional aspect as birds consume water at twice the level of feed. Various factors such as the microbial level, pH, mineral content, hardness, or organic matter load determine the quality of water and each of these should be within an acceptable range to ensure the quality of water. Unless the quality of supplied drinking water to poultry is guaranteed, achieving the growth and feed efficiency potential provided by intensive genetic selection, ideal grow-out environments and optimal nutrition programs becomes a challenge. In many cases, poultry farms experience poor flock performance or health related issues in several flocks for no obvious reasons and often the issues are traced to the water supply. Therefore, it should be of primary concern for production personnel and poultry producers to know the quality of water supplies provided to their birds and confirm if the parameters are within acceptable ranges and free of any undesirable contaminants. Water supplies such as wells or reservoirs are dynamic with water quality changing as often season to season. Establishing routine testing of supplies and taking corrective action when necessary can have a significant impact on optimizing flock performance. While the introduction of enclosed water systems such as nipple drinkers during the early 1990's revolutionized the industry by dramatically improving water quality, unfortunately the industry became complacent with water system sanitation, primarily because this type of system removed water supplies from being visually inspected and created a sense of "out of sight, out of mind" mentality. Since then more has been learned about biofilms and their role in creating microbial populations which survive and thrive within water lines and drinker systems and create health challenges that are not easily addressed.

The goal of poultry water sanitation procedures and sanitizer/disinfectant products is to target microbial challenges that exist and thrive in water supplies whether they are bacterial, fungal, viral or protozoal. Chlorine products, most commonly sodium hypochlorite or calcium hypochlorite, have been the primary water disinfectant products for thirty-forty years in the poultry industry. Unfortunately, microbes are becoming resistant to these products because they have not been always used properly. Therefore, the industry needs to identify other options and have clear guidelines on the efficacy of alternatives as well as optimal usage levels. The best sanitation practice combined with an efficacious product is essential for maintaining desired water quality for optimum flock health and performance. Therefore, two different projects aimed at enhancing the microbial quality of drinking water were conducted. The first study was conducted to determine the value and impact of water sanitation practices on microbial loads in water supplies and water lines. A second in vitro trial was conducted to evaluate hydrogen peroxide products, an alternative oxidizing disinfectant to chlorine for daily water sanitation, for residual and efficacy over time.

CHAPTER I: Review of Literature

1. Water Needs for Poultry

Water is the most important nutrient and is physiologically required by all animals including poultry. Therefore, the quantity and quality of water should be supplied on a daily basis as per the bird age and breed to keep all physiological functions intact. Furthermore, a daily and per cycle water consumption by commercial birds is regarded as a prime indicator from a health and welfare perspective [1]. So, besides the production perspective, providing adequate and good quality water is listed as a basic animal welfare criterion [2-4]. The total content of water in a bird averages from 65-70% of its lean body mass [5, 6] and water consumed by birds is generally utilized for nutrient transportation, body temperature regulation, joint lubrication and various intra and extracellular biochemical reactions.

1. 1. Water Consumption

Various factors such as ambient temperature [7, 8], humidity and air velocity [9], feed intake [10], dietary formulation [11, 12], drinking water presentation [13-15], age and sex [16], and genetics [17] govern the amount of daily water intake. Besides these factors, properties of water like water temperature [18, 19] and levels of minerals and contaminants [20, 21] also affect the consumption of water and the overall performance of birds. High water consumption is correlated with optimal feed to gain ratio [22].

Genetic research in the poultry industry, especially in the breeding sector, is an ongoing process with the goal of better performance by improving the breed lines through intense selection. Improved selection strategies result in enhanced production traits in birds such as

growth rate [23, 24], feed efficiency [25, 26] and yield [27]. These production attributes are not obvious unless the physiology of the birds is altered [28, 29] and are sometimes accompanied by negative complications [30] or undesired traits [31] as well. To avoid the negative complications from selection pressures and to capture the full genetic potential, existing husbandry practices need to be reviewed accordingly. Energy requirements and therefore the water requirements should be reconsidered for every cross bred progeny. The significant increase in water consumption by birds of today as compared to birds reared in the past has already been reported. In 2010-2011 birds drink 5.5 gallons more on day 7 and 13 gallons more on day 42 per 1000 birds as compared to birds that were reared a decade earlier [32].

2. Water Quality: Microbiological Aspect

Water is presumed safe if it has a zero microbial population, provided that mineral content is at safe levels and undesired contaminants are not present. However, presence of microbes in water is not always correlated with a disease in flocks unless it increases above a certain infectious level. The following table gives the acceptable levels of bacteria in colony forming units (cfu) per milliliter (ml) in drinking water for poultry operation [33, 34].

Table 1. Drinking Water Quality Standards for Poultry

source	good	maximum acceptable	unacceptable
Main water supply	<100	< 300	> 300
Total aerobic plate counts	0	<1000	>1000
Total coliforms	0	50	>50
Fecal coliforms	0	0	1
<i>E. coli</i>	0	0	1
<i>Pseudomonas</i>	0	0	1

If the source water has an acceptable bacterial level, it does not mean the levels present at the end of drinker line where the birds are drinking is also within safe microbial levels. The following field evaluations demonstrate how the microbial levels can significantly change by the time the water supply reaches the end of the drinker system from the source, if the drinker system is unhygienic [33].

Table 2. Aerobic Bacteria Levels in Drip Samples (cfu/ml)

farms	at source	at end of lines
A	2,700	26,600
B	600	282,000
C	0	4,775,000

Microbial contamination above the acceptable levels in drinking water directly affects health and performance [35]. The microbial problem with *E. coli* and *Pseudomonas* in water was found similar in top and bottom producing farms [36] indicating water as a vulnerable source for microbial contamination regardless of good management. Similarly, some farms have experienced campylobacteriosis in chickens, which were caused by water borne *Campylobacter jejuni* [37]. Reduced broiler performance was recorded in water contaminated with coliforms and

Enterobacter, with more aggravated conditions detected when accompanied by elevated nitrate-nitrogen contamination [38, 39].

Furthermore, poultry specific endemic pathogens like *Campylobacter* easily thrive in poultry drinking water [40] and drinker lines act as a potential source of *Campylobacter* colonization in chickens [37,41, 42]. Coliforms like *E. coli* are readily found in fecal contaminated well water [43] and are associated with the cases of colibacillosis. *Salmonella* infections in chickens have been traced from various water sources [44, 45] including water tanks, drinkers [46] and water samples in poultry units [47]. So, water treatment has been suggested as a control strategy for salmonellosis at the farm level [48, 49]. Avian influenza strains that cause high mortality in poultry and are capable of causing flu pandemics in humans can persist for long periods of time in water [50]. Similarly, water contamination through viruses in feces can lead to viral diseases such as infectious bursal disease and avian encephalomyelitis. Protozoal diseases like histomoniasis and coccidiosis can also be transmitted by contaminated water [51]. Testing and treating water can help reduce potential microbial contamination issues.

2. 1. Biofilms: The Slime that Build up in Water Systems

The US poultry industry has adopted an enclosed drinking water system which is less vulnerable to microbial contamination than the open type bell or trough drinker. Salmonellosis has been detected in several farms in other parts of the world that do not use an enclosed system [51, 52]. Furthermore, an enclosed system has an advantage of holding higher disinfectant residuals [53]. However, the use of an enclosed system is only a partial solution and biofilms can still develop in waterlines over time with low or no disinfectant residual level in water even if the water supplies are clean [54]. Non sanitized water systems can harbor high levels of biofilms in water lines that not only foul the water [55] but also limit water availability to birds [56].

Biofilms are complex communities of a matrix of different species of enclosed microbial cells cooperating with one another for survival and are firmly attached to hydrated surfaces [57, 58]. Microorganisms that form biofilms are different from their free-living counterparts in terms of growth rate, composition and show increased level of resistance to biocides which may be attributed to their up regulation and down regulation of different genes [59, 60].

Biofilm buildup or inactivation in water systems is affected by factors like disinfectant classes and their efficacies [61, 62], pipe materials used [63-66], water temperature [67, 61] and water flow rate [68, 69]. Disinfectants available on the market have different efficacies to control biofilms under dehydrated and hydrated conditions. So, the true efficacy of any disinfectant can only be revealed if tested against biofilms grown in fluid -flow conditions [70].

Biofilms corrode the pipe material and deteriorate the water quality besides providing ecological niches for better survival of pathogens [71]. The material used in poultry water lines, polyvinyl chloride (PVC), easily forms biofilms that harbor diverse microbes including food borne pathogens [72, 73]. Poultry specific biofilms promote the entrapment and survival of pathogens like *Campylobacter* [74]. Opportunistic pathogens like *Pseudomonas* can easily thrive in poultry waterlines, and line cleaning with appropriate disinfectants at effective concentration is strongly suggested [75,76].

Wholesomeness of water and water systems are not possible without addressing biofilm problems. Practicing regular water sanitation and line cleaning between flocks can solve much of the microbial problem in water including biofilm buildup in water systems [75]. Poultry operations performing daily water sanitation and which also conduct line cleaning between flocks have improved performance [77]. Furthermore, practices of water system sanitation and provision of safe water to birds are effective hygiene barriers to minimize poultry contamination and transmission of enteric foodborne pathogens to humans [78, 48].

3. Disinfectants for Water System Sanitation

Disinfection is the main part of an effective biosecurity program in poultry operations to prevent entry of disease agents and foodborne pathogens in birds [79, 80]. Ideal disinfectants used as a drinking water sanitizer should create disinfectant residuals throughout the distribution system and should inactivate microbes, control biofilms or neutralize undesired contaminants. The Environmental Protection Agency (EPA) has listed the following characteristics (Table 3) in

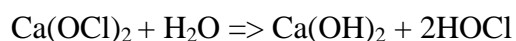
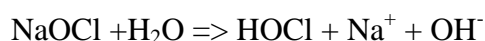
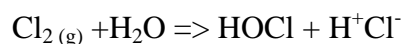
disinfectant residuals as ideal in drinking water for humans [81]. These also hold true for drinking water disinfection/sanitation in animals as well.

Table 3. Water Treatment Desired Characteristics

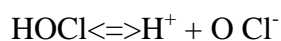
Chemical
Easily measured on-site under field conditions
Minimal to no interferences with common constituents in drinking water
Generates minimal to no disinfection by-products
Long-lasting
Selectively reactive (minimal to no corrosion/reaction with dissolved metals, pipe materials, linings, etc.)
Operational/Physical
Highly soluble in water
Safely generated, transported, stored, and fed
Cost-effective relative to the application (large- or small-scale)
Inactivation Capabilities
Effectively and efficiently inactivates wide range of organisms (bacteria, viruses, protozoa, algae, fungi)
Effectively inactivates microorganisms present in the bulk water and those associated with particles/biofilm
Achieves desired level of organism inactivation at doses that are safe for human consumption
Aesthetic
Achieves desired level of organism inactivation without creating tastes and odors
Overfeed can be detected by taste, odor, and/or color

Chemically, water disinfection is carried out using powerful oxidizers such as chlorine [82] and oxygen/reactive oxygen species [83], or by using heavy metal ions such as silver and copper [84-86], or in synergism with the oxidizers and heavy metals [87,88]. Physically, it is carried out by using ultraviolet rays [89-91] and ultrasonic [92, 93]. Though each class of disinfectants act specifically against microbes, their general biocidal activity can be explained by their ability to oxidize or rupture the cell wall of microorganisms or to diffuse into cells and interfere with the cellular metabolism [94, 95]. In the case of viral agents, permanent disruption in capsular proteins or nucleic acids occurs [96]. Increased efficacy is attained by cleaning away organic matter and then applying the disinfectant [97]. At higher concentrations, most disinfectants act in random and non-specific ways against microbes [98].

In poultry operations, the commonly used disinfectants/oxidizers for drinking water sanitation are sodium hypochlorite, chlorine gas and calcium hypochlorite [99,100] which when present in the optimal pH range will create hypochlorous acid on hydrolysis [82].

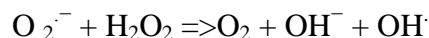


Hypochlorous acid has a strong germicidal action. However, in high pH conditions (>8.5 pH), it dissociates completely into hypochlorite ions which has a less germicidal action than the hypochlorous acid. The pH range between 6.5 and 8.5 has incomplete dissociation, while pH below 6.5 has no or a negligible dissociation of the hypochlorous form [82,101].



Chlorination is more effective at lower pH levels [102] and often drinking water is acidified to support chlorine disinfectant efficacy for improved sanitizing residual which supports better bird performance [103]. However, careful selection among various acid products available is necessary to avoid water consumption impacts [104]. When using chlorine and acidifiers together in water, they should be mixed and injected separately to avoid poisonous gas formation [105].

The use of chlorine sanitizer in high pH water [101,102], or in weaker concentrations [106,107], or in water systems with well-established biofilms [108], the sanitizing value of chlorine is greatly reduced. Therefore, the poultry industry needs to identify other options and have clear guidelines on the efficacy of alternative disinfectants as well as their optimal usage levels. Recent field experiences have shown that poor performing farms are greatly benefitted from water sanitation programs using hydrogen peroxide as an alternative disinfectant to chlorine [109]. Hydrogen peroxide inactivates microbes creating oxidative stress by forming very strong oxidizing agents, hydroxyl radicals, from superoxide (O_2^-) radicals [110], and readily oxidizes the proteins and microbial enzymes; however, efficacy differs between liquid and gaseous forms [111].



3. 1. Chlorine and Hydrogen Peroxide as Water System Sanitizers

When drinking water has 2-5 ppm free chlorine residual, it is effective against most microbial growth in water [34]. Adding chlorine in drinking water showed increased livability in birds [112]. Chlorine levels below 50 ppm in drinking water are well tolerated by birds; above 50

ppm, impacts on water intake and production performances are detected with toxic level developing at 200 ppm [21,113,114].

Disinfectant residual levels required for the microbial inactivation vary according to the nature of water quality. Within a minute of contact time in drinking water, significant reductions in *E. coli* O157:H7 isolates and H5N1 virus were observed at 0.25 and 0.52-1.08 ppm of free chlorine levels, respectively. [115,116]. The disinfection strategies with 0.2 to 0.4 ppm of free chlorine in drinking water showed promising results in farms with *Campylobacter* challenges in chicken flocks [37] whereas, there were controversies in its effectiveness at even higher concentrations than this level for *Campylobacter* inactivation [107], indicating each case could be different depending upon the type of water used. Chlorine in drinking water 12-hours prior to slaughter helped in disinfecting the crop and ceca of broilers and reduced the *E. coli* and *Enterococci* load [117].

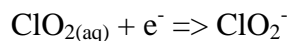
Another effectively used water sanitizer is hydrogen peroxide. Maintaining 25-50 ppm of hydrogen peroxide residuals in the water is considered the Effective Residual Concentration (ERC) [118]. Stabilized hydrogen peroxide products hold higher concentrations of residuals for a longer time than non-stabilized [76,119]. This disinfectant at 3% has a rapid bactericidal effect and is effective against a wide range of viruses, yeast, and fungi [120]. Successful cleaning of poultry waterlines with hydrogen peroxide products with minimal equipment damage can be done [121].

The use of various concentrations of hydrogen peroxide has been studied for their antimicrobial efficacies in both human and animal research. A solution of 0.03 % hydrogen peroxide demonstrated effective results in controlling *E. coli* and *Salmonella* load in fruit juices [122]. The use of hydrogen peroxide at 0.5% in flushing human dental water lines improved the water quality over time by effectively reducing heterotrophic bacterial counts below 200 cfu/ml [123]. 2 % hydrogen peroxide for a 3 hour contact time [124] and 3 % dilution for a 1 hour of contact time showed effective antimicrobial activity against *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Aspergillus fumigatus* and *Fusarium* species with organic matter present [125]. Heavy metal ions like silver and copper, and organic acids like peracetic and ascorbic acid in hydrogen peroxide synergize the disinfecting property of hydrogen peroxide [87,126-129] particularly in heavily contaminated water [130].

When disinfectants act on biofilms, their efficacy against the microbial species in biofilms is greatly reduced as compared to their efficacy against planktonic counterparts due to their limited penetrability into the biofilm matrix [108]. The degree of resistance of biofilms against disinfectants differs with the microbial species constituting them and with disinfectant types and concentration used. Chlorine based and peroxide based disinfectants performed well in inactivating *Pseudomonas aeruginosa* [131] and *Listeria monocytogenes* [132] biofilms. For Infectious Laryngotracheitis (ILT) virus biofilms in water line, hydrogen peroxide had comparative effectiveness as compared to chlorine [133]. Furthermore, hydrogen peroxide also acts as a surface disinfectant and is effective against *Salmonella* and *Staphylococci* biofilms [46,134,135]. Similarly, an aqueous solution at 0.88 mol/liter of hydrogen peroxide for a 6 hour contact time is effective against bacterial spores in surface application [136].

3. 2. Other Water Sanitizers in Poultry Operation

Another successfully used water disinfectant for sanitizing poultry drinking water is chlorine dioxide. It acts as a selective oxidant as it has a single electron transfer mechanism and reduces to form chlorite ion which exists as the dominant species in water [82].



If the water is dirty or has a significant organic load, then disinfecting with chlorine requires higher free residuals of chlorine thus impacting taste and odor. Sanitizing with chlorine dioxide is a good option [33] because its use in similar water supplies does not cause the taste or odor issues. Chlorine dioxide kills bacteria and viruses similar to or better than chlorine and is unaffected by a wide pH range [137,138].

Other disinfectants like quaternary ammonium compounds and iodophores are also used in poultry operation for disinfecting water and water system [139,140].

As oxidizing agents are generally used during water disinfection, Oxidation Reduction Potential (ORP) values give the oxidizing ability of the chemicals in water to oxidize/kill microbes. The ORP values are affected by concentration of oxidizing residuals and are pH dependent [102, 141,142] and 650 mV or above in water is considered enough to destroy most bacteria and viruses within few seconds [142].

Secondary oxidant functions of disinfectants in water include oxidation of iron and manganese [143] which helps to minimize drinker coagulation [33,144], and maintaining the

biologically safe and stable environment in water thereby preventing the regrowth of microbes, algal blooms and biofilm formation in the water distribution systems [145].

4. Hypothesis

Literature review exhibits that microbial hygiene of water and water system in poultry operation is one prime requirement for ensuring bird health and optimizing performance. Cleaning of drinker lines in between flocks and practice of regular water sanitation using appropriate disinfectant at effective concentration can solve much of the microbial problems in water including the biofilm buildup in water systems. Based on this assumption, two separate projects, both aimed at enhancing the microbial quality of poultry drinking water were conducted. The first study was conducted to determine the value and impact of water sanitation practices on microbial levels in water supplies and water lines. A second in vitro trial was conducted to evaluate hydrogen peroxide products for residual and efficacy over time, as an alternative oxidizing sanitizer to chlorine for daily water sanitation.

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CHAPTER II

IMPACT OF WATER SYSTEM SANITATION PRACTICES ON MICROBIAL LEVELS IN WATER SUPPLIES OF BROILER HOUSES

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SUMMARY

An evaluation of poultry farm water supplies was conducted to determine the value and impact of water system sanitation practices in commercial broiler houses on microbial levels. Four barns of a commercial poultry unit that sanitize water systems by daily water sanitation practice (providing 0.5 ppm to 1 ppm of free chlorine residual or above 600 mV of Oxidation Reduction Potential (ORP) in water at the beginning of water lines) and line cleaning between flocks using a concentrated disinfectant were selected. Regular drip samples (at various time intervals during bird grow-out to cover entire flock period) and swabs samples (pre-flush, post flush and Day 43 when bird were not present) were taken to examine microbial levels in water and water lines from all four barns for three consecutive flocks. Drip and swab samples taken during birds present from different farms that did not clean lines between flocks and did not practice water sanitation were also evaluated. Cleaning water lines with a strong disinfectant solution and flushing the lines before placing chicks significantly reduced ($P < 0.05$) the aerobic plate count (apc) levels compared to the levels that existed prior to flushing ($< 1 \log_{10} \text{ cfu/ml}$ versus $> 4 \log_{10} \text{ cfu/ml}$). This evaluation showed water is vulnerable to microbial contamination regardless of regular water sanitation and therefore occasional microbial surges were noticed during different points of flock grow-out period. Practicing daily water sanitation controlled the occasional

microbial surges in water from persisting and kept drinking water at a microbiologically safe level (<1000 cfu/ml). Bacterial biofilms, with significantly higher counts ($> 4 \log_{10}$ cfu/ml) than post flush counts ($P<0.05$) and not differing from their pre flush counts ($P> 0.05$), reoccurred in water lines by the 6th week of bird grow-out, regardless of daily water sanitation. Microbial results from untreated farms revealed water systems with a significantly higher level ($P < 0.05$) of apc, yeast and mold (6.63, 3.84 and 2.42 \log_{10} units cfu/ml, respectively) in swab samples than their corresponding drip samples indicating drip samples alone could not represent the overall sanitation status of the water system. Since the bacterial biofilms can still develop in water lines over time regardless of line cleaning and daily water sanitation usage, this evaluation suggests that line cleaning between flocks is an effective practice to optimize water system hygiene for the next flock of chicks.

Key words: water sanitation, microbial levels, disinfection

DESCRIPTION OF PROBLEM

Providing ad libitum access to clean and safe drinking water to poultry is a basic requirement for optimizing production. One prime factor that determines the wholesomeness of drinking water is its microbial quality. Microbial contamination above the acceptable levels in drinking water can directly affect health and performance [1]. Water is vulnerable to microbial contamination. Both the top and bottom producing farms suffer equally from microbial contamination like *E. coli* and *Pseudomonas* [2]. Health and production related issues in birds including breeders have been reported in various farms due to poor microbial water quality [3-7]. Fecal contaminated well water is a source of coliforms like *E. coli* that cause colibacillosis in poultry flocks [8]. Water and water systems including water tanks and drinker lines act as potential sources for *Salmonella* and *Campylobacter* (including viable but non-culturable forms) in chickens [7, 9-11] and water treatment is a control strategy at the farm level [12,13].

The introduction of enclosed water systems such as nipple drinkers during the early 1990's revolutionized the industry by dramatically improving water quality. Unfortunately the industry became complacent with water system sanitation, primarily because this type of system removed water supplies from being visually inspected and created a sense of "out of sight, out of mind" mentality. Since then more has been learned about biofilms and their role in creating microbial populations which survive and thrive within water lines and drinker systems creating health challenges that are not easily addressed. The material used in poultry water lines, polyvinyl chloride (PVC), easily forms biofilms that harbor the diverse microbes including foodborne pathogens [14, 15]. Furthermore, biofilms create ecological niches that allow better survival of pathogens [16] and promote pathogen entrapment [17]. It limits the water availability to birds by clogging drinker lines and over time corrodes them. Practice of regular water

sanitation and line cleaning between flocks can solve much of the microbial problem in water systems including biofilm development [18]. Poultry operations performing daily water sanitation and line cleaning between flocks have improved performances [19].

This study was conducted with the objective of determining the value and impact of water system sanitation practices in commercial broiler houses on microbial levels in water supplies and water lines.

MATERIALS AND METHODS

Study barns

A four barn commercial poultry farm that cleans waterlines between flocks and practices a daily water sanitation program was chosen. The size of each barn was 40 by 400 feet with a 20,000 market- age bird rearing capacity.

Each barn contained eight separate waterlines (four running 185 feet on either side of the feed line in each half of the barn). Internal diameter of the lines was $\frac{3}{4}$ inch and the pipes were constructed of poly vinyl chloride (PVC).

Cleaning of water lines

The water line cleaning was performed between flocks using electrolyzed water containing primarily chlorine, but also chlorine dioxide, ozone radicals, and chlorite as disinfectants in a highly concentrated solution (>1000 ppm of chlorine residuals in water) and was allowed to sit for 24 hours before flushing from the lines with water that contained 1-2 ppm of chlorine. The lines were flushed again within 24 hours of chick placement.

Water sanitation practice

Daily water sanitation was conducted by adding chlorine to drinking water which provided a free chlorine residual of .5 to 1 ppm in the beginning portion of water lines.

Collection of swab samples

A total of eight pre-flush and eight post flush swab samples, two from each of the four barns, were taken using standard swabbing techniques. Different lines were used for taking pre-

flush and post flush samples. A second set of eight samples were taken (two from each of the same four barns but taken from different lines than the ones used for pre-flush and post flush lines) with birds absent on day 43 at the end of grow-out cycle. Different lines were used since it was assumed that once a line was swabbed, then re-swabbing a line that had already been swabbed might not yield the same results as a line which had not had the biofilm already disturbed by swabbing. These sampling procedures were repeated for three consecutive flocks for the same 4 barns from the farm.

The swabbing technique involved utilizing sterile swabs placed in a vial of 25 ml of sterile Butterfields Phosphate Diluent (BPD). First step of the procedure required the water supply to the drinker line to be turned off. Next the end cap was removed from the drinker line and the line was drained completely. Alcohol wipes were used to sterilize tweezers as well as the outside rim of the pipe on the drinker line. Forceps were flamed for five to ten seconds to burn off excess alcohol and to further sterilize the instrument. Utilizing the forceps, the sponge was grasped within the opened swab vial (with 25 ml of BPD) and the forceps then compressed the sponge in order to squeeze the excess BPD from the sponge prior to removing the sponge from the vial. After the sponge was removed from the vial with the forceps, it was carefully inserted into the end of the water line, taking care to touch the sponge to only the inside of the line. Next the sponge was inserted approximately 3-4 inches (6-10 cm) into the line and then turned in a clockwise rotation so that the sponge gently wiped the entire inside circumference of the pipe. The sponge was returned to the vial, the cap tightly screwed back onto the vial and then the vial was placed on an ice pack for transport to the lab for microbial enumeration.

While the microbial results for samples collected using the swab technique do not represent the exact number of cells present in the biofilm of the particular area sampled inside the line, it do provide a good estimate of the sanitary condition inside the drinker lines.

Collection of drip samples

Two drip samples were collected from two of the eight lines from the end nipple on each line in each of the four barns using a sterile technique. Sterile forceps were used to activate the nipple drinker so that water dripped down from the drinker tip into a sterile whirl pack bag. Approximately 20 ml of water was collected before sealing the bag. This procedure was conducted in all four barns on five different occasions in Flock 1 and Flock 3 and on seven different occasions in Flock 2 with the sample days throughout the life of each flock. Immediately upon collection, drip samples were packed in ice and transported to the laboratory for microbial analysis.

Introduction of citric acid and laryngotracheitis (LT) vaccine additives on microbial water quality

For Flock 3, citric acid was introduced into the water system on day 5 (454 gram packet was mixed into 2 gallons of water to prepare the stock solution, then this was administered at a rate of 1:128 into the water system) until day 8 at which time it was combined with vaccine stabilizer (sodium thiosulfate based product at 25 grams mixed to 18.16 gallons of water) on day 6 and then both were given in combination with the modified live laryngotracheitis vaccine (LT) on day 7 followed by only chlorination on day 8 and onward. Water system sampling was done to determine the impact on the microbial quality of the drinking water by collecting two drip and

two swab samples per barn on day 10. Drips were also collected on day 15 in order to observe if any changes in microbial levels occurred in the water due to daily water sanitation program.

Collection of water samples from untreated barns

A total of 19 drip samples and 19 swab samples (from corresponding water lines to drip samples) were collected from 19 barns from different commercial broiler farms which did not clean/sanitize water lines between flocks or when birds were present and did not practice water sanitation during flock grow-out period.

Measurement of free chlorine, pH and ORP for treated barns

Measurements of free chlorine, pH and Oxidation-Reduction Potential (ORP) were conducted at different locations on the water line systems: the anteroom (where the water supply entered the barn), at the beginning of drinker line and at the end of the drinker line. These measurements were done a day prior or during the day before taking drip sampling. The distances between anteroom and beginning, and beginning and end of the line were approximately 200 feet and 185 feet respectively. The free chlorine and pH were measured using Pocket Colorimeter TM II Cat. No. 58700-12 from Hach test kit. ORP was measured using Oakton ORPTestr10" Eutech Instruments, serial number 1537652. Approximately 50 milliliters of water was collected at each site and the tests were performed within 10 minutes of water collection.

Microbial plating

Microbial plating was carried out for the collected swab and drip samples for aerobic plate count (apc), and yeast and mold count using 3MPetrifilmTM. One milliliter of water was placed on the Petrifilm. Serial dilutions were performed by diluting one ml in 9 ml of sterile water and then spinning the solution for 10 seconds. Enumeration of microbes was carried out after 48 hours of incubation at 30°C for apc and after 72 hours of sitting at room temperature (20°C) for yeast and molds.

Results analysis

All microbial counts were converted to log10 prior to analysis to normalize data distribution. Results were analyzed using the GLM procedure of SAS [20] with sanitation practice and barn serving as the main effects. Results which were significant at the $P < 0.05$ level were separated using the least square means procedure.

RESULTS AND DISCUSSIONS

1. Pre, post flush and day 43 swab results

The average log₁₀ values of aerobic plate count (apc) in colony forming units (cfu) per milliliter (ml) before and after flush in water lines for the Flocks 1 and 2 and after flush for the Flock 3 are shown in Table 1. In Flock 1, pre-flush average log₁₀ apc of 4.763 (standard error (SE) of 0.45 log₁₀) units was observed in water lines for the barns in Flock 1. Post-flush values dropped to 1.56 log₁₀ (SE= 0.45 log₁₀) which was significantly less than the pre-flush counts ($P < 0.05$). In Flock 2, the apc pre-flush log₁₀ was 3.43 units (SE= 0.34 log₁₀) which was significantly lower than the pre-flush value in Flock 1 ($P < 0.05$). Again, post flush apc counts in Flock 2 dropped to 0.98 log₁₀ which was almost a three log reduction that was significantly different from pre flush levels ($P < 0.05$). Similar results were observed in post-flush count in Flock 3 where the average post flush count was 1.05 log₁₀ (SE= 0.27 log₁₀).

Day 43 apc, yeast and mold counts for all four barns for the Flocks 1 and 3 are presented in Table 2. Significant increases in the day 43 apc swab levels were observed for flocks 1 and 3 as compared to initial post flush swab results obtained prior to flock placements (4.40 log₁₀ and 4.13 log₁₀ versus 1.56 log₁₀ and 1.05 log₁₀ respectively for Flocks 1 and 3 ($P < 0.05$). These findings indicate that biofilm development can reoccur even in the presence of a daily water sanitizer. Yeast and mold counts averaged a log₁₀ value of one in both the flocks. These results indicate that yeast and mold do not appear to have the same biofilm reoccurrence rates as the apc biofilm under the current water system management strategy.

2. Microbial results for untreated barns

Table 3 gives the microbial status of the drip and corresponding swab samples of water lines taken from barns that did not practice any form of water system sanitation either between flocks or when birds were present.

The apc, yeast and mold counts (6.63 log₁₀ (SE=0.28 log₁₀), 3.84 log₁₀ (SE = 0.51 log₁₀) and 2.42 log₁₀ (SE = 0.37 log₁₀)) in the swab samples in untreated water lines were significantly higher (P<0.05) than the waterline samples taken at day 43 for Flocks 1 and 3 for treated barns. These swab results were also significantly higher than their corresponding drip results (P< 0.05) whose log₁₀ counts were 2.98 (SE= 0.30 log₁₀), .51 (SE =0.42 log₁₀) and 0.18 (SE= 0.39 log₁₀) for apc, yeast and mold respectively.

3. Impacts on LT vaccine procedure on microbial water quality

Table 4 shows the effect of the LT vaccine procedure on microbial levels of water lines and Table 5 shows daily water sanitation impact on microbial water quality after LT vaccine procedure.

There was a significant increase in microbial count (apclog₁₀ average= 6.33 units (SE= 0.46 log₁₀); mold log₁₀ average= 3.59 (SE = 0.55 log₁₀)) in water lines after LT vaccine procedure were introduced in water as compared to post flush microbial data (P< 0.05). The microbial count in drip samples spiked and were too numerous to count (tntc) per ml of sample at 3rd order of one- tenth serial dilutions indicating the average counts being above 5.36 log₁₀ (SE= 0.7

log₁₀) However, microbial results from day 15 in drip samples showed that daily sanitation practice dropped counts significantly ($P < 0.05$) to 1.38 log₁₀ (SE= 0.7 log₁₀).

After LT vaccine procedure in water for day 10 sampled, significant drops in free chlorine levels in water at different locations of water line system were noticed ($P < 0.05$). Initially it had an average residual of 0.6 mg/L (SE = 0.12mg/L) in anteroom which dropped to 0.36 mg/L (SE= 0.12 mg/L) in the beginning of line and then to 0 mg/L. Drops were also noticed in ORP levels from anteroom to beginning ($P < 0.05$), and from beginning to the end of lines reaching below 500 mV at the end of the lines.

4. Drip microbial results

The apc levels in drip samples remained generally within the acceptable range (< 1000 cfu/ml) with yeast and mold being insignificantly present or absent for all barns and for all flocks during flock grow-out period. All barns exhibited similar pattern of microbial growth for different samples days. Occasional surges in microbial levels were noticed during the flocks but did not persist for longer days.

Flock 1: Microbial samplings were conducted on days 2, 11, 18, 25 and 43 during the bird grow-out period. Day 2 and Day 43 counts had average log₁₀ units of 4.14 and 4.27 cfu/ml respectively and were significantly higher from counts in other days at day 11, 18 and 25 ($P < 0.05$) where the apc levels remained within acceptable range for the barns. Yeasts and mold after day 2 were also very low or absent in other sampling days.

Flock 2: Microbial samplings were conducted on days 5, 8, 19, 26, 29, 40 and 43 during bird grow-out period. Day 40 had significant apc surge (log₁₀ unit of 4.47 cfu/ml) than counts of other days plated (fairly zero cfu/ml) for the barns but this surge was not observed on day 43 samples. Yeast and mold counts remained less than a log unit cfu/ml or were absent throughout the flock period.

Flock 3: Microbial samplings were done on days 10, 15, 19, 30 and 42 during bird grow-out period. Significant microbial surges influencing water quality (average apc 5.47 log₁₀ units in each barn) were noticed in day 10 following the actual LT vaccination procedure at day 7. On day 15, there was a significant drop ($P < 0.05$) in the microbial levels to acceptable microbial ranges except for barn 2 which showed persistently high levels of microbes for day 15 and day 19 sampled (free chlorine readings were less than 0.2 ppm for that barn for day 10, 15 and 19). For other days sampled, microbial populations were absent or were within the acceptable ranges.

5. Readings of free chlorine, oxidation reduction potential (ORP) and pH

Fluctuations in free chlorine residuals and ORP levels in different locations of water line system for the different sampled days during the flock grow-out period were noticed for all barns and all flocks. Co-related patterns of lower readings of free chlorine residuals (< 0.2 ppm) and ORP levels (< 600 mV) were noticed with microbial surges occurring in water during the sampled days for all barns. However, for all sampled days with lower levels of free chlorine residuals or ORP levels noticed, microbial surges were not observed.

Significant free chlorine reductions were noticed from anteroom to beginning and from beginning to end of the lines for average values taken combining days and barns in each flock ($P < 0.05$). However, the average free chlorine levels maintained at the beginning of lines were 0.63 ppm (SD=0.07 ppm), 0.67 ppm (SD=0.04 ppm) and 0.81 ppm (SD = 0.10ppm) for Flock 1, 2 and 3 respectively during the bird grow-out period and the end residual levels approaching approximately 0.5 ppm in all flocks (figure 10). Average ORP levels maintained fairly steady pattern from anteroom to beginning and from beginning to end of the lines with slightly decreasing trend from start to end location (Figure 11) in each flock. Average ORP levels measured in Flock 1 and 2 were well above 600 mV both at the beginning and the end of the lines whereas Flock 3 had the readings below 600 mV in both the locations (beginning and the end of lines) with the average reading being affected by the initial low readings due to LT vaccine procedure. The pH levels recorded in water for all sampled days throughout all the flocks were 7.0 ± 0.2 .

Disinfecting water and water supplies and controlling microbiological issues related to water is taken as an important measure to minimize water borne diseases in broiler production [21]. Higher concentration of disinfection acts in random and non-specific ways [22]. Nevertheless, lower disinfection residual concentration is preferred as long as it is effective as it minimizes hazardous disinfectant byproducts formation. Though the birds can tolerate 50 ppm of chlorine without adverse effects [23, 24], maintaining 2-5 ppm of free chlorine residuals in water is adequate to effectively control most microbial growth [25]. In humans, maximum residual disinfectant level of chlorine in drinking recommended by EPA is 4mg/L [26]. Unlike other disinfectants, chlorine acts in a specific way depending upon the type of microbes and

environment of water. Within a minute of contact time in drinking water, significant reductions of *E. coli* O157:H7 isolates at 0.25 ppm and H5N1 viral strain at 0.52-1.08 mg/L free chlorine levels were respectively noticed [27, 28]. The disinfection strategy with 0.2 to 0.4 ppm of free chlorine in drinking water has shown promising result in farms with *Campylobacter* problems in chickens [5].

When oxidizing agents like chlorine are used during water disinfection, Oxidation Reduction potential (ORP) values give the oxidizing ability of the disinfectant residuals in water to oxidize/kill microbes. The ORP values are affected by concentration of oxidizing agents and are pH dependent [29-31] and 650 mV or above in water destroys most bacteria and viruses within a few seconds [29].

Microorganisms that form biofilms are different from their free-living counterparts in terms of growth rate and composition and show increased resistance to biocides which may be attributed to their up regulation and down regulation of different genes [32, 33]. When disinfectants like chlorine act on biofilms, their efficacy against the microbial species in biofilms is much more reduced as compared to effectiveness against planktonic counterparts due to chlorine's limited penetrability into the biofilm matrix [34]. So, manufacturer recommended doses may not work with established biofilms [35]. However, 1 mg/l of free residual chlorine in water inactivated the biofilm grown in PVC [36]. Opportunistic pathogens like *Pseudomonas* can easily thrive in waterlines and line cleaning with appropriate disinfectant at an efficacious rate is necessary [37, 38]. Strict cleaning and disinfecting of drinker systems and provision of safe

water to birds are effective hygiene barriers to minimize poultry contamination and transmission of enteric foodborne pathogens in humans [11, 39].

CONCLUSIONS AND APPLICATIONS

- Line cleaning between flocks and maintaining daily water free chlorine level between 0.5 and 1 ppm at the beginning of water lines (ORP levels of 600 mV or above) helps keep microbial levels in water within the acceptable range (< 1000 bacterial cfu/ml) during the bird grow-out period. However, at these levels, biofilm buildup with high microbial levels ($>4 \log_{10}$ cfu/ml) can still occur in water lines over time. Therefore, it is mandatory to clean lines between flocks to optimize water system hygiene for next flock of chicks.
- Even with a consistent sanitation program, residual levels in water in water line systems can fluctuate by locations and by time during the flock which could be a result of fluctuations in water quality and flow rates. Therefore a consistent monitoring program is essential for optimal success with water sanitation procedures.
- This evaluation showed that water is subject to fluctuations in microbial levels with random spikes of unacceptable levels which can occur at any time during the bird grow out period regardless of regular water sanitation. However, these microbial surges do not persist long if daily water sanitation is in present.
- Water lines can be heavily contaminated with biofilm even if the drip samples are within acceptable microbial levels, and could shed at any point of time in water supplies posing greater health risk especially for young chicks or immune-compromised birds.

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TABLE 1: Aerobic plate counts (log₁₀ cfu/ml) associated with swabs taken with pre and post water line flushing for three consecutive flocks

	sample	Flock 1		Flock 2		Flock 3
		pre flush*	post flush*	pre flush*	post flush*	post flush
Barn 1	1	5.02531	1.62325	1.92428	1.63347	0.30103
	2	4.74036	1.36173	4.53147	2.99123	2.10732
Barn 2	1	4.9345	0.77815	3.04532	0.60206	0
	2	4.63347	0.69897	3.50514	1.20412	0
Barn 3	1	4.10037	0.60206	4.77085	0	0.77815
	2	4.08636	0.60206	3.63347	1.11394	1.79239
Barn 4	1	4.08636	6.57978	3.65321	0	1.51851
	2	6.50379	0.30103	2.39794	0.30103	1.86332
Average		4.76381	1.56837	3.43271	0.98073	1.04509

* Pre flush and post flush counts in flock 1 and flock 2 differ significantly (P< 0.05).

TABLE 2. Microbial levels (log₁₀ cfu/ml) for water line swabs collected at day 43 from farms that treated the water systems

	Flock 1			Flock 3		
	apc	yeast	mold	apc	yeast	mold
Barn 1	4.77815	0	0	3.50718	0	0
Barn 2	4.41497	0	0.95424	5.01703	0	0
Barn 3	4.24551	0	0.60206	3.62273	0	0
Barn 4	4.17319	0	0	4.37749	1.64345	1
Average	4.402955	0	0.389075	4.131108	0.410863	0.25

TABLE3. Microbial counts (log10 cfu/ml) associated with swab and drip samples taken from untreated water lines during birds present

	Swab* results			Drip* results		
samples	apc	yeast	mold	apc	yeast	mold
1	6.8954	.	.	1.51815	.	.
2	2.86332	2.17609	2.32222	2.63347	.	.
3	4.25527	1.51851	0.30103	2.54407	0	0
4	5.53148	4.95904	0	3.32015	0	1.477121
5	7.14301	tntc**	tntc	1.59106	0	0
6	7.16435	3.27875	3.14613	2.5682	0	0
7	7.08636	1.8451	1.47712	.	.	.
8	7.13672	2.14613	0	1.875061	0	0
9	7.17898	0	2.65321	4.27646	0	0.477121
10	7.0607	4.92942	3.30103	0	0	0
11	7.14301	tntc	tntc	5.40933	2.792392	0
12	6.98677	5.15534	3.69897	2.77085	0	0
13	7.08636	5.77	0	2.70757	0	0
14	7.00432	tntc	0	4.12057	1.770852	0
15	6.89763	4.27	4.17609	2.70757		0
16	7.14301	tntc	.	4.43136	3.176091	0
17	7.24797	tntc	0	4.33846	.	0
18	7.25042	0	1.23045	2.15836	.	0
19	7.02938	tntc	tntc	3.73239	.	0.778151
Average	6.637077	3.848639	2.420956	2.987949	0.51494	0.170775

*P values for apc, yeast and mold are <0.05 for drip and swab comparison

**tntc= too numerous to count at 3rd serial dilution of one-tenth dilution and are replaced with 4.47 log10 for calculating average considering 300 as the maximum countable cfu/ml in a dilution

**TABLE 4. Impact of LT vaccine procedure on broiler drinking water microbial levels
(log10 cfu/ml) as determined by swabbing the water line**

	Before* LT vaccine procedure			After* LT vaccine procedure		
	apc	yeast	mold	apc	yeast	mold
Barn 1	1.875	0	0	6.93952	0	3.3979
Barn 2	0	0	0	7.07918	0	3.8692
Barn 3	1.531	0	0	4.94939	0	1.6435
Barn 4	1.724	0	0	6.38021	.	5.4771
Average	1.2825	0	0	6.337075	0	3.596925

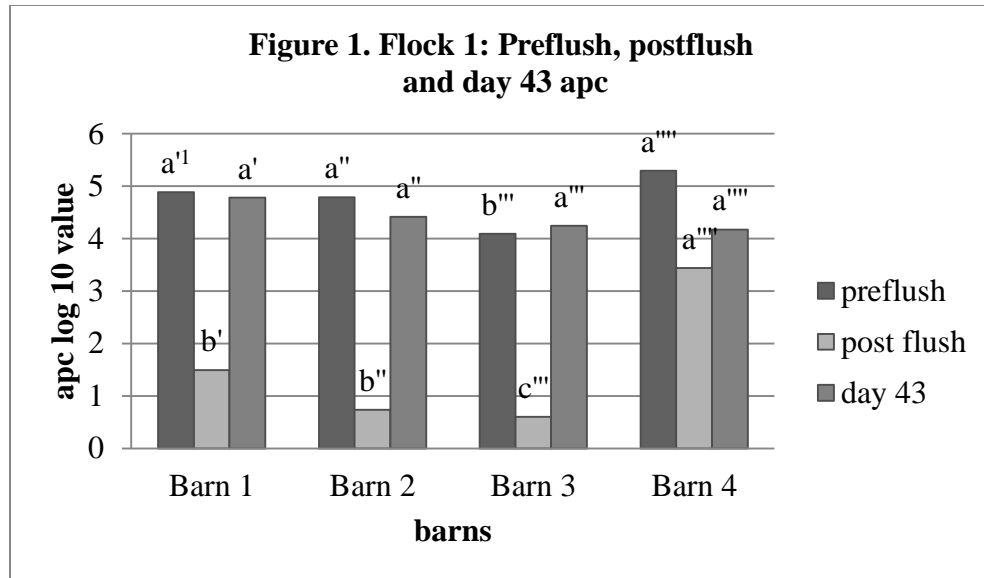
*P values for apc and mold before and after differ significantly (< 0.05)

TABLE 5. Daily water sanitation impacts after of LT vaccine procedure on drinking water quality as determined by drip sampling (log10 cfu/ml)

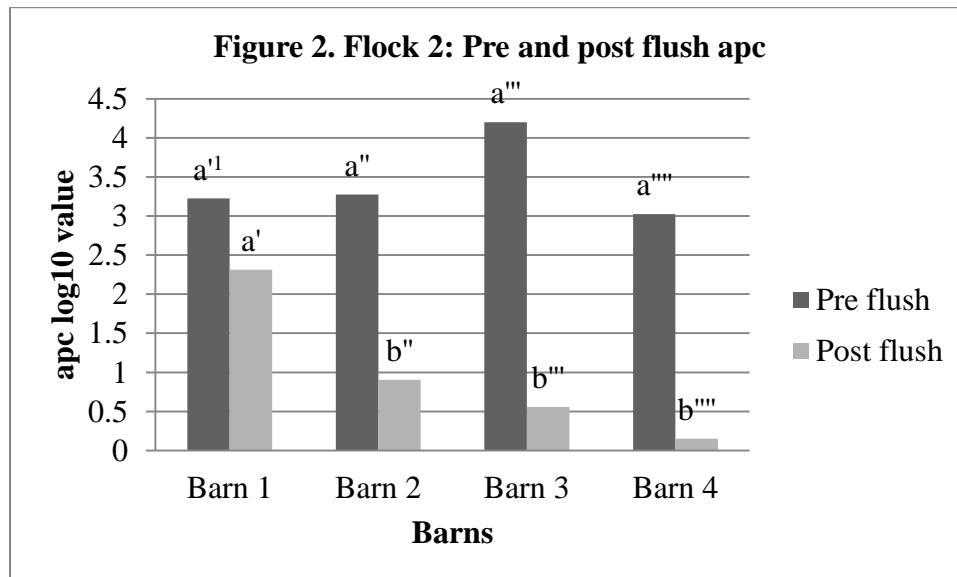
	On day 10*			On day15*		
	apc	yeast	mold	apc	yeast	mold
Barn 1	tntc **	0	4.47	0	0	0
Barn 2	tntc	1.81954	1.30103	4.20194	0	2.31597
Barn 3	tntc	0	2.3222	1.34242	0	0
Barn 4	5.04532	2.04139	4.47	0	0	0
Average	5.36383	0.965233	3.140808	1.38609	0	0.578993

*P values for apc, yeast and mold on day 10 and on day 15 differ significantly (<0.05)

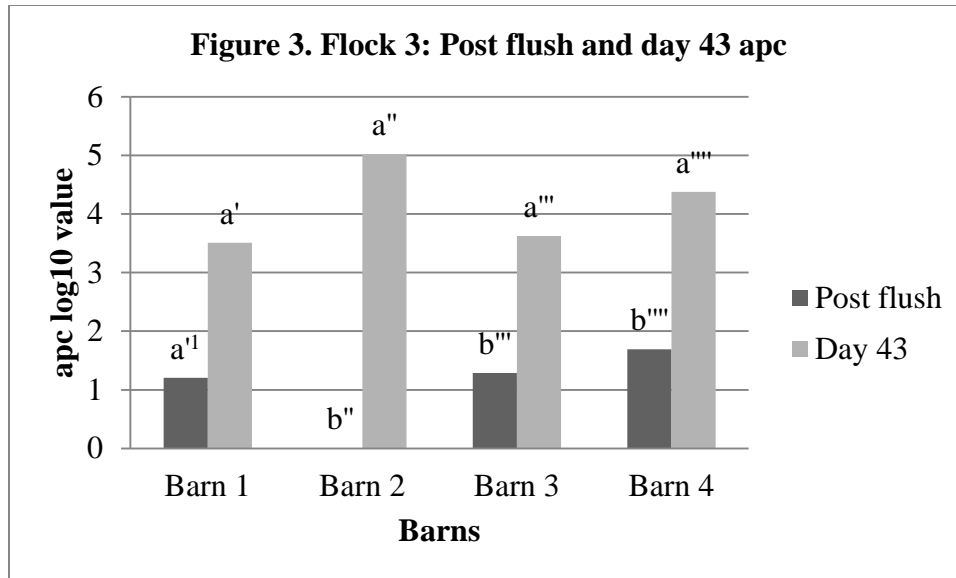
**tntc= too numerous to count at third serial dilution of one-tenth dilution and are replaced with 5.47 log10 for calculating average considering 300 as the maximum countable cfu/ml in a dilution



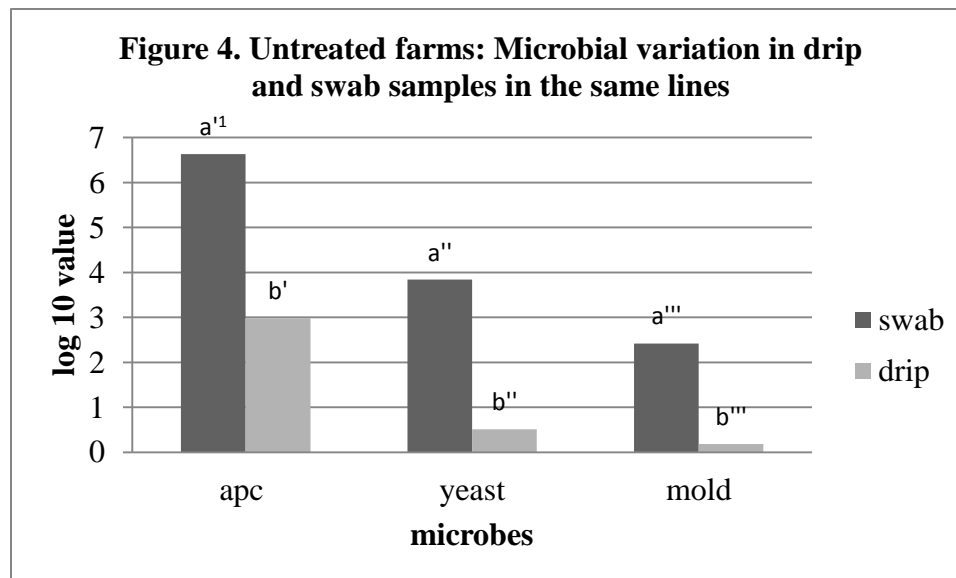
1. Barn means with different letters are significantly different ($P < 0.05$)



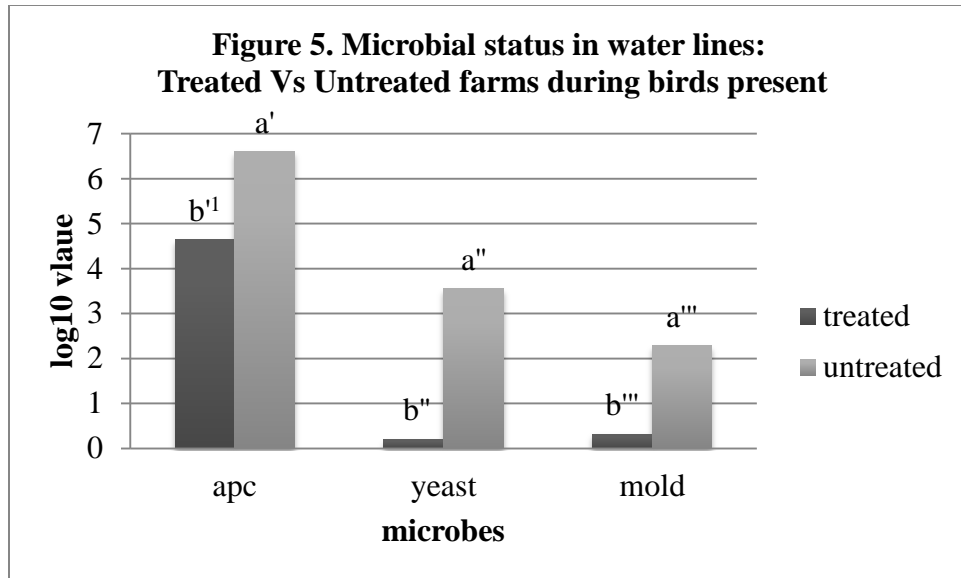
1. Barn means with different letters are significantly different ($P < 0.05$)



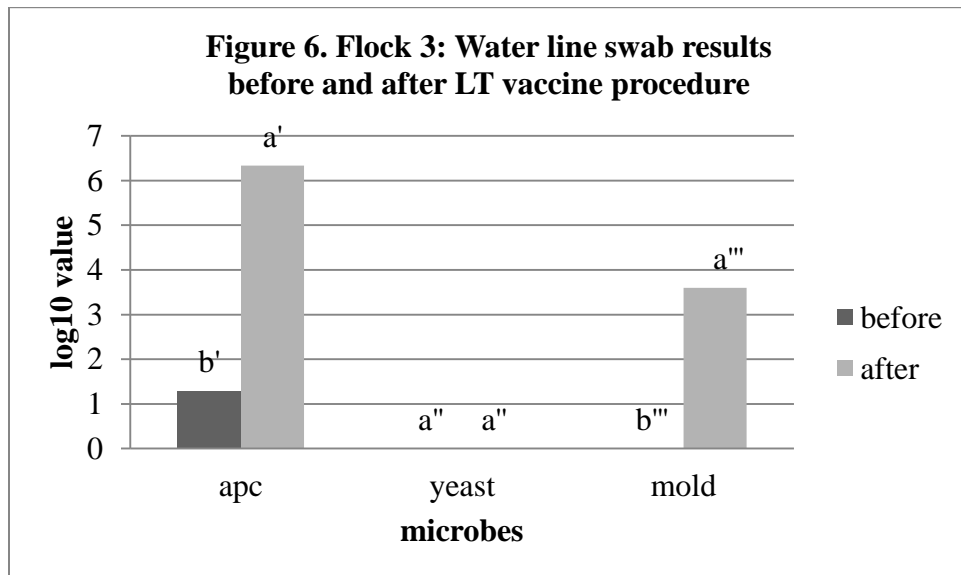
1. Barn means with different letters are significantly different ($P < 0.05$)



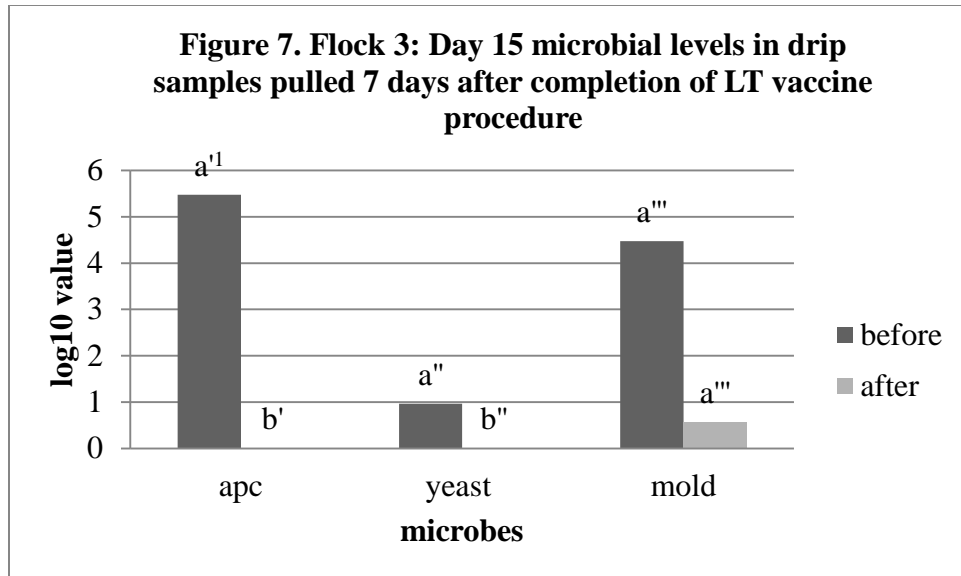
1. Means with different letters are significantly different for apc, yeast and mold ($P < 0.05$)



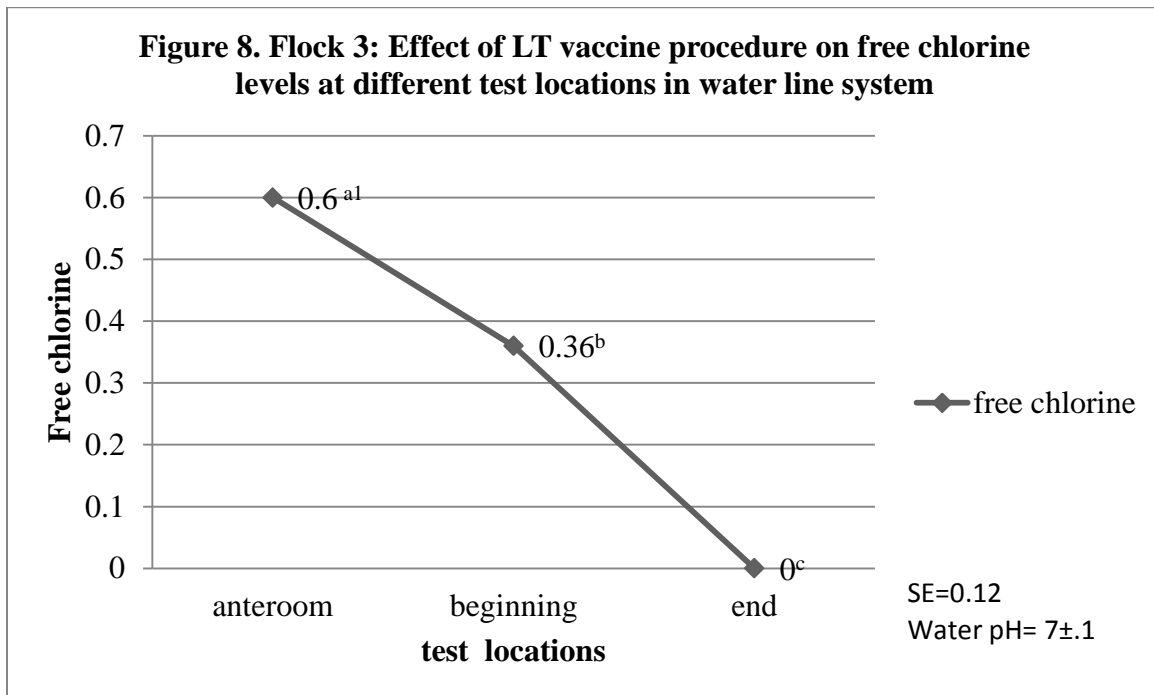
1. Means with different letters are significantly different for apc, yeast and mold ($P < 0.05$)



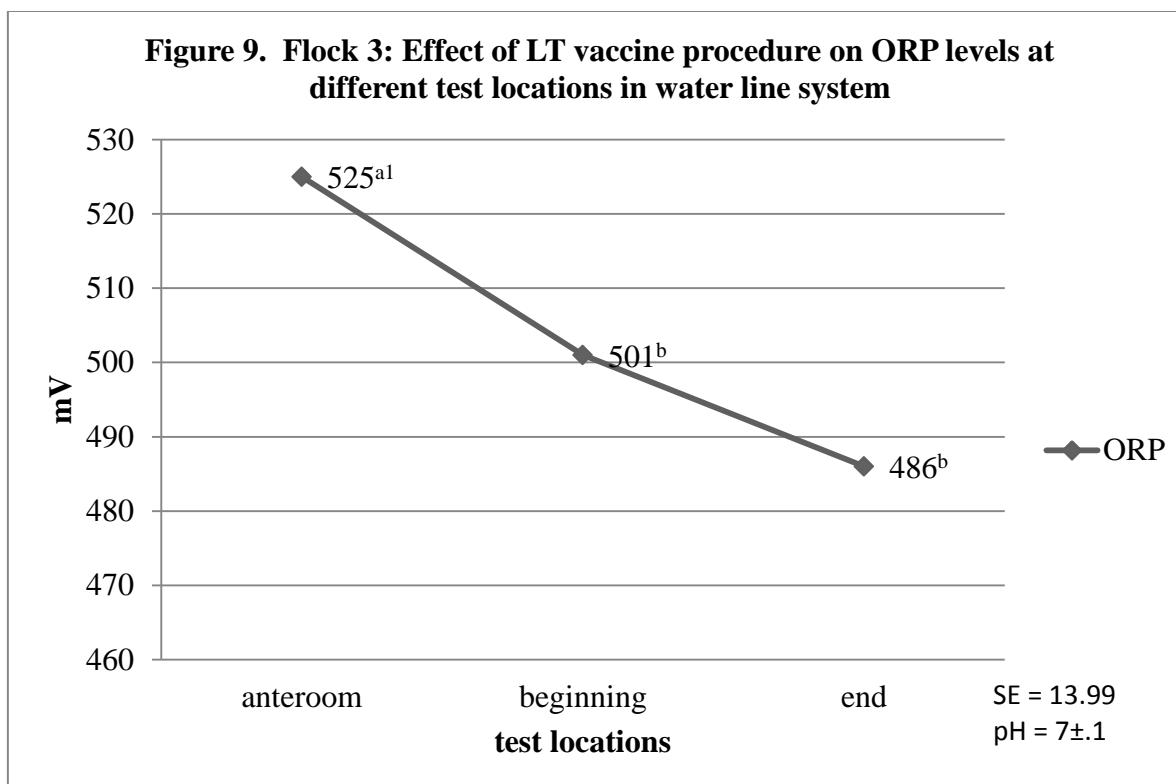
1. Means with different letters are significantly different for apc, yeast and mold ($P < 0.05$)



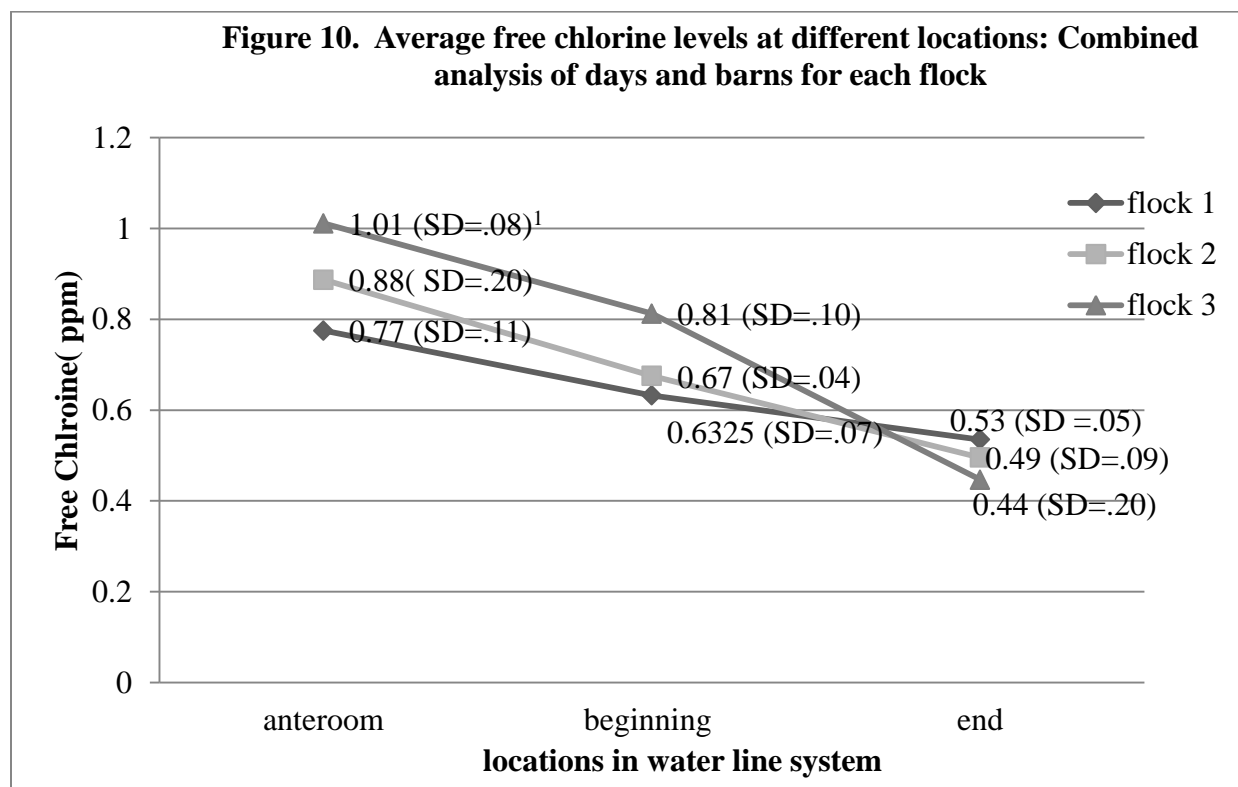
1. Means with different letters are significantly different for apc, yeast and mold ($P < 0.05$)



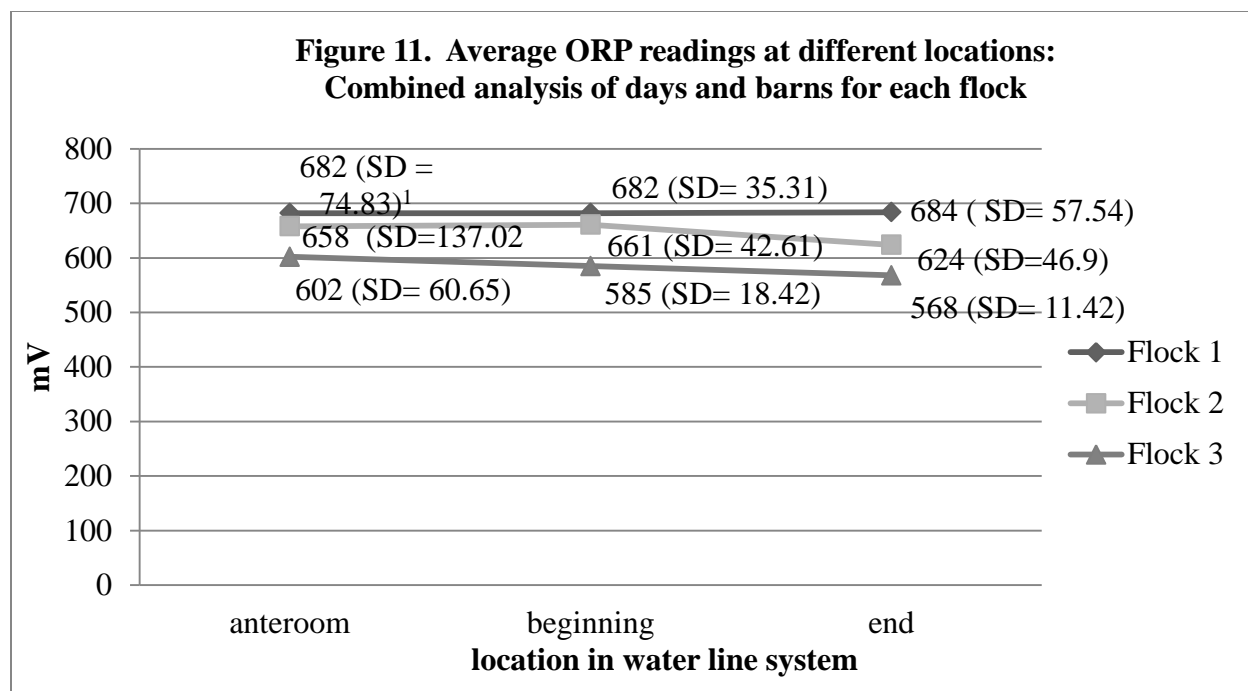
1. Means with different letters are significantly different ($P < 0.05$)



1. Means with different letters are significantly different ($P < 0.05$)



1. Significant drop ($P < 0.05$) : Anteroom to beginning and from beginning to end in each flock



1. No significant differences ($P > 0.05$) exist in the locations for the all three flocks.



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To The University of Arkansas Graduate School:

Please accept this letter as an acknowledgement that Pramir Maharjan did the majority of the work described in the manuscript entitled “Impacts of water system sanitation practices on microbial levels in water supplies of broiler houses” which is part of his thesis that is entitled “Evaluation of water sanitation options for poultry production”.

Sincerely,

Susan Watkins, PhD

Professor and The Arkansas Poultry Federation Chair of Poultry Science

CHAPTER III
EVALUATING DIFFERENT HYDROGEN PEROXIDE PRODUCTS FOR RESIDUAL
AND EFFICACY OVER TIME

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Primary Audience: Growers, Producers, Nutritionist, Researchers

SUMMARY

Four commercially available hydrogen peroxide products were tested for residuals and efficacy over time. Each product was added at the rate of 59.14 ml, 118.28 ml and 177.42 ml per 3780 ml of water creating stock solutions. Test solutions that actually mimic the bird drinking rate were made from each stock solution mixing at the rate of 29.57 ml of stock solution added to 3780 ml of water. Residual activities of test solutions prepared were measured from day 0 to day 5. Forty-eight hours post treatment, a 5 ml aliquot of water with a heavy microbial load was introduced into the test solutions as challenge and microbial plating for aerobic bacteria and mold was done for zero and one hour contact times. Results of this experiment suggest that an Effective Residual Concentration (ERC) of 25-50 ppm in test solution starts at two ounces of stock solution for all products evaluated. Stabilized products stay at the higher residual level and can maintain ERC for a longer time than non-stabilized products. Significant bacterial reductions ($P < 0.05$) within an hour of contact time can be achieved at concentrations of 59.14 ml of stock solution or lower for all products provided that the ERC is maintained. Higher residuals or longer contact time are required for mold control.

Key words: hydrogen peroxide, residuals, water, efficacy

DESCRIPTION OF PROBLEM

The poultry industry understands the value of clean and sanitized water supplies for optimizing bird performance and reducing the costs associated with grow-out. Disinfecting water with chlorine for human drinking purpose has been a century old practice in the US [1] and is considered as the standard practice of water sanitation in animal husbandry as well. Nevertheless, the use of chlorine sanitizer in a high pH of water [2, 3], or at weaker concentrations [4, 5], or when the water systems have well established biofilms [6], results in a significant reduction in the sanitizing efficacy of chlorine. In commercial production barns, newly hatched chicks and poults are provided water supplies that are warmed to prevent chilling the birds. It has been documented that chicks less than a week old drink 5-10 gallons per thousand birds in a 24 hour period [7]. This small volume of water usage means water often remains in waterlines for several hours. This results in loss of efficacious chlorine residuals which could leave birds vulnerable to microbial challenges from biofilms. It is of high interest to the industry to identify alternative water sanitizers which could remain efficacious for extended periods of time.

Recent field experiences have shown that poor performing poultry farms are greatly benefitted from a water sanitation program utilizing hydrogen peroxide (H_2O_2) products [8]. Maintaining 25-50 ppm of hydrogen peroxide residuals in the water is considered as the Effective Residual Concentration (ERC) [9]. There are numerous sources of H_2O_2 products available for poultry water system sanitation and their concentration ranges from 20 %- 50 % with or without stabilizers. The industry/grower practices the use of those products without actually monitoring the residuals.

Therefore, this study was conducted with the objective of determining baseline information on different H₂O₂ products prepared at different concentration levels for residual activities over time. To measure how effective these solutions were in limiting or reducing microbial growth when challenged with heavy laden microbial water was the second study goal.

MATERIALS AND METHODS

An in vitro experiment was carried out to evaluate different hydrogen peroxide products for residuals and efficacy over time.

Hydrogen Peroxide Products

Four commercially available hydrogen peroxide products commonly used in poultry drinking water disinfection system were obtained for evaluation.

1. Product A- 50% H_2O_2 with silver complex
2. Product B- 20 % H_2O_2 with peracetic acid mixture
3. Product C- 34 % H_2O_2
4. Product D-28 % H_2O_2

Products A, B and C were stabilized whereas product D was not.

Water Used

Municipal water was used for preparing the stock and test solutions for the trials. Before the water was used for preparing the solutions, it was allowed to sit for 48 hours in open container to dissipate the chlorine residual.

Preparation of Stock and Test Solutions

Each product was added at the rate of two, four and six ounces (59.14 ml, 118.28 ml and 177.42 ml per gallon (3780 ml) of water creating stock solutions and then final mixtures as test solutions were made from each stock solution by mixing an ounce (29.57 ml) of stock solution added to a gallon of water. For this in vitro evaluation, one ml of each stock solution prepared

was pulled and added to 128 ml of water. These test solutions actually mimic the medicator injection rate of 1:128 that is commonly used for adding water products to the drinking water. Each test solution and the control without any treatment were replicated thrice and the trial was repeated once. After the solutions were prepared they were covered to prevent sunlight access, except during the residual measurement and microbial plating.

Residual Measurement

Peroxide residuals were measured for each test solution from day 0 to day 5 in both the trials. In trial 1, the residual measurement was carried out using Water Works test strips that measure from less than 0.5 ppm to 100 ppm. In trial 2, Mini Analyst Series 942 Hydrogen Peroxide meter was used and provided a more precise measurement of the peroxide residual.

Challenge Introduction and Microbial Plating

At 48 hours post treatment, a 5ml aliquot of microbial water was added as challenge (apc bacterial log₁₀ values - 4.2 and 5.7 in trial 1 and trial 2 respectively; mold log₁₀ values: 3.0 and 3.07 in trial 1 and trial 2 respectively) was introduced to two replicates of each of the treatments and two replicates of control. A third replicate of each treatment and control were kept challenge free. Microbial plating were then carried out for aerobic plate count (apc) and mold count at 0 hour and 1 hour post challenge introduction using PetrifilmTM. Enumeration of microbes was carried out after 48 hours of incubation at 30 °C for apc and after 72 hours of sitting at room temperature for molds.

Result Analysis

All microbial counts were converted to log₁₀ prior to analysis to normalize data distribution. Results were analyzed using JMP Pro 10 software using one way analysis of ANOVA [10]. Statistical means for significant differences were considered for $P < 0.05$.

RESULTS AND DISCUSSIONS

Residual Results

The average residual activities of different hydrogen peroxide products for trial 1 and trial 2 over days are presented in Table 1 and Table 2 respectively.

In both the trials, Product A maintained a higher peroxide residual level followed by product C while product D remained the lowest among all 4 products at each concentration level from day 0 to 5. However, product D at the 2 ounces stock solution concentration level maintained the lower limit of ERC of 25 ppm until day 1 in trial 1 and until day 2 in trial 2. The residual activity of product D was significantly lower ($p < .05$) than all other stabilized products A, B and C when it started to drop off below the ERC at this concentration level. Other stabilized products A, B and C at the 2 ounces stock solution concentration level maintained ERC at least a day more than non-stabilized product D. In trial 2, at 4 and 6 ounces stock solution concentration levels, stabilized products A, B and C were above the ERC all days throughout the trial period. Even the non- stabilized product, D, maintained the peroxide residual above the ERC at 6 ounces concentration level till day 5.

Microbial Results

Trial 1

The results of aerobic plate count and mold count at 0 hour and 1 hour post challenge introduction for trial 1 are presented in Table 3 and Table 4.

Immediately after the challenge introduction (at 0 hour contact time) on day 2, there were significant reductions in bacterial count ($P < 0.05$) with all the products at all concentration levels

as compared to the control. At the 1 hour post inoculation interval, there was again a reduction by a log with respect to the count values observed at the 0 hour contact time for all the products and at all concentration levels. An important thing to note was there were no significant differences in bacterial reduction within the product at 2, 4 and 6 ounces concentration levels for all products at both 0 and 1 hour contact time although there were significant differences in their residual activities in these levels. Mold reductions were found to be significant ($P < .05$) only at 6 ounces concentration level by an hour of contact time for all products.

Trial 2

The results of aerobic plate counts and mold counts at 0 hour and 1 hour post challenge introduction for trial 2 are presented in Table 5 and Table 6.

Only product B at all concentration levels (2, 4 and 6 ounces) gave significant reductions ($P < 0.05$) in bacterial counts at 0 hour contact time than the control. However, by one hour of contact time, all products at all concentration levels dropped the bacterial count to a significantly lower ($P < 0.05$) level as compared to the control. An important point to note again was there were no significant differences in bacterial reduction within the product at 2, 4 and 6 ounce stock solution concentration levels for all products at both the 0 and 1 hour contact times although the residual activities did vary significantly at these levels. Mold counts were found to be significantly lower for stabilized products A, B and C only at 6 ounces stock solution concentration level by an hour of contact time.

In both the trials, none of the products at any concentration level tested completely eliminated the microbes by one hour of contact time.

Hydrogen peroxide (H_2O_2) has a strong oxidizing property against biomolecules and its oxidizing property and efficacy are greatly affected by the formulation and physical state [11]. Compounds like silver and peracetic acid in hydrogen peroxide have shown to synergize with the disinfecting property of hydrogen peroxide [12-15].

The use of various concentrations of hydrogen peroxide has been studied for their antimicrobial efficacies in both human and animal research. A solution 0.03% hydrogen peroxide proved effective in controlling *E. coli* and *Salmonella* load in fruit juices [16] whereas 2 % hydrogen peroxide for 3 hour contact time [17] and 3 % solution for an hour of contact time had complete antimicrobial activity [18]. Hydrogen peroxide acts as surface disinfectant and is effective against the biofilms such as of *Salmonella* and *Staphylococci* [19-21].

In previous studies conducted at the University of Arkansas, different stabilized and non-stabilized hydrogen peroxide products were evaluated for residuals and efficacy over time and had similar results [22, 23].

CONCLUSIONS AND APPLICATIONS

1. Effective Residual Concentration (ERC) of hydrogen peroxide in drinking water starts at 2 ounces per gallon of stock solution for all the products evaluated. At this rate, non-stabilized product maintain ERC for 2-3 days whereas stabilized products maintain longer (at least one day more) than stabilized.
2. One hour of contact time is adequate to reduce the bacterial load significantly under the high challenge condition, provided that the ERC is maintained. Residual activities of hydrogen peroxide in water above the ERC (of 25-50 ppm) do not have better bacterial control.
3. Higher concentrations or longer contact time are required for mold control.
4. Disinfecting the water with these products at 4 and 6 ounces per gallon of water to make stock solutions leave higher residuals than ERC for several days. Studies can be carried out for the maximum tolerable residuals the chicks/birds can drink without health compromise.

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TABLE 1. Trial 1: Average Residual Activity (in ppm) of Different Hydrogen Peroxide Products over a 5 Day Period

Products, Concentration	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
Product A, 2oz/gal	>100 ^a	>50 ^d	>50 ^d	25 ^h	<25 ⁱ	>10 ^j
Product B, 2oz/gal	50 ^e	<50 ^f	<25 ⁱ	10 ^k	<10 ^l	>5 ^m
Product C, 2oz/gal	50 ^e	<50 ^f	25 ^h	>10 ^j	<10 ^l	<10 ^l
Product D, 2oz/gal	50 ^e	25 ^h	<25 ⁱ	<10 ^l	>5 ^m	<5 ^o
Product A, 4oz/gal	>100 ^a	>100 ^a	100 ^b	50 ^e	<50 ^f	>25 ^g
Product B, 4oz/gal	>100 ^a	<100 ^c	50 ^e	25 ^h	<25 ⁱ	<25 ⁱ
Product C, 4oz/gal	<100 ^c	50 ^e	<50 ^f	>25 ^g	>25 ^g	25 ^h
Product D, 4oz/gal	<100 ^c	50 ^e	<50 ^f	25 ^h	>10 ^j	>10 ^j
Product A, 6oz/gal	>100 ^a	>100 ^a	>100 ^a	>100 ^a	100 ^b	<100 ^c
Product B, 6oz/gal	>100 ^a	>100 ^a	100 ^b	>50 ^d	<50 ^f	>50 ^d
Product C, 6oz/gal	>100 ^a	100 ^b	<100 ^c	>50 ^d	50 ^e	<50 ^f
Product D, 6oz/gal	>100 ^a	<100 ^c	50 ^e	>25 ^g	>25 ^g	>25 ^g

^{a-o}Means with different superscripts are significantly different (P < 0.05).

TABLE 2. Trial 2: Average Residual Activity (in ppm) of Different Hydrogen Peroxide Products over a 5 Day Period

Products, Concentration	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5
Product A , 2 oz/gal	79.0 ^e	76.7 ^e	64.2 ^{gh}	58.6 ^{hijk}	55.5 ^{klm}	>50 ^{lmn}
Product B , 2 oz/gal	44.4 ^{op}	37.1 ^{pq}	32.9 ^s	27.0 ^{tu}	26.3 ^u	>10 ^w
Product C , 2 oz/gal	53.5 ^{klm}	49.6 ^{mn}	41.2 ^{pqr}	36.5 ^{qrs}	32.6 st	>10 ^w
Product D , 2 oz/gal	36.3 ^{rs}	34.1 ^s	26.6 ^{tu}	22.1 ^{uv}	19.2 ^v	>10 ^w
Product A , 4oz/gal	>100 ^a	>100 ^a	>100 ^a	100.5 ^a	98.7 ^{ab}	<100 ^{ab}
Product B , 4oz/gal	83.1 ^e	77.2 ^e	67.5 ^{fg}	58.8 ^{hijk}	57.6 ^{ijkl}	>50 ^{lmn}
Product C , 4oz/gal	98.3 ^{ab}	94.9 ^{bc}	77.6 ^e	67.6 ^{fg}	63.1 ^{ghi}	50.0 ^{mno}
Product D , 4oz/gal	70.2 ^f	70.4 ^{fg}	55.8 ^{ijkl}	45.2 ^{nop}	45.1 ^{nop}	<50 ^{mno}
Product A , 6 oz/gal	>100 ^a	>100 ^a	>100 ^a	>100 ^a	>100 ^a	>100 ^a
Product B , 6 oz/gal	>100 ^a	>100 ^a	97.5 ^{ab}	88.0 ^d	88.0 ^d	<100 ^{ab}
Product C , 6 oz/gal	>100 ^a	>100 ^a	>100 ^a	>100 ^a	98.2 ^{abc}	<100 ^{ab}
Product D, 6 oz/gal	99.7 ^{ab}	93.2 ^{cd}	76.7 ^e	60.8 ^{hij}	57.8 ^{ijk}	>50 ^{lmn}

^{a-w} Means with different superscripts are significantly different (P < 0.05).

TABLE 3. Trial 1. Aerobic Plate Count (log₁₀ cfu/ml)

Products, Concentration	0 hour	1 hour
Product A, 2oz/gal	3.84 ^{bc}	2.61 ^g
Product B, 2oz/gal	3.52 ^{de}	2.62 ^g
Product C, 2oz/gal	3.72 ^{cd}	2.61 ^g
Product D, 2oz/gal	3.72 ^{bcd}	2.71 ^g
Product A, 4oz/gal	3.53 ^{de}	2.63 ^g
Product B, 4oz/gal	3.23 ^f	2.72 ^g
Product C, 4oz/gal	3.90 ^{bc}	2.66 ^g
Product D, 4oz/gal	3.74 ^{bcd}	2.65 ^g
Product A, 6oz/gal	3.45 ^{ef}	2.63 ^g
Product B, 6oz/gal	3.29 ^{ef}	2.74 ^g
Product C, 6oz/gal	3.71 ^{cd}	2.56 ^g
Product D, 6oz/gal	3.71 ^{cd}	2.76 ^g

^{a-g} Means with different superscripts differ significantly (P < 0.05).

TABLE 4. Trial 1. Mold Count (log10 cfu/ml)

Products, Concentration	0 hour	1 hour
Product A, 2oz/gal	1.00 ^{defg}	1.13 ^{abcdef}
Product B, 2oz/gal	1.23 ^{ab}	1.21 ^{abc}
Product C, 2oz/gal	1.09 ^{bcdef}	1.12 ^{abcdef}
Product D, 2oz/gal	1.19 ^{abcd}	1.08 ^{bcdef}
Product A, 4oz/gal	0.84 ^{ghi}	0.92 ^{fgh}
Product B, 4oz/gal	1.15 ^{abcde}	1.15 ^{abcde}
Product C, 4oz/gal	1.10 ^{bcdef}	1.02 ^{cdefg}
Product D, 4oz/gal	1.04 ^{bcdefg}	0.95 ^{efgh}
Product A, 6oz/gal	0.69 ^{ijk}	0.54 ^k
Product B, 6oz/gal	1.02 ^{bcdefg}	0.59 ^{jk}
Product C, 6oz/gal	1.11 ^{abcdef}	0.69 ^{ijk}
Product D, 6oz/gal	1.16 ^{abcde}	0.75 ^{hij}
Control	1.14 ^{abcde}	1.31 ^a

^{a-k} Means with different superscripts differ significantly(P < 0.05).

TABLE 5. Trial 2. Aerobic Plate Count (log₁₀ cfu/ml)

Products, Concentration	0 hour	1 hour	24 hour
Product A, 2oz/gal	5.60 ^{ab}	4.93 ^{efgh}	2.77 ^{lm}
Product B, 2oz/gal	5.23 ^{cde}	3.21 ^k	2.77 ^{lm}
Product C, 2oz/gal	5.27 ^{bcd}	5.09 ^{defg}	2.87 ^{lm}
Product D, 2oz/gal	5.69 ^a	5.10 ^{defg}	3.04 ^{kl}
Product A, 4oz/gal	5.56 ^{abc}	4.72 ^{hij}	2.87 ^{lm}
Product B, 4oz/gal	5.12 ^{def}	2.69 ^m	2.65 ^{mn}
Product C, 4oz/gal	5.74 ^a	4.81 ^{fghij}	2.77 ^{lm}
Product D, 4oz/gal	5.59 ^{ab}	4.79 ^{ghij}	2.73 ^{lm}
Product A, 6oz/gal	5.60 ^{ab}	4.49 ^j	2.76 ^{lm}
Product B, 6oz/gal	4.52 ^{ij}	2.69 ^m	2.33 ⁿ
Product C, 6oz/gal	5.69 ^a	4.73 ^{hij}	2.74 ^{lm}
Product D, 6oz/gal	5.62 ^a	4.83 ^{fghi}	2.84 ^{lm}
Control	5.79 ^a	5.75 ^a	5.87 ^a

^{a-n} Means with different superscripts differ significantly (P < 0.05).

TABLE 6. Trial 2. Mold Count (log10 cfu/ml)

Products, Concentration	0 hour	1 hour	24 hour
Product A, 2oz/gal	0.94 ^{abc}	0.87 ^{abcdef}	0.48 ^{ghi}
Product B, 2oz/gal	0.92 ^{abcd}	0.92 ^{abcd}	0.35 ⁱ
Product C, 2oz/gal	0.70 ^{cdefg}	0.90 ^{abcde}	0.81a ^{bcdef}
Product D, 2oz/gal	0.95 ^{abc}	0.93 ^{abcd}	0.70 ^{cdefg}
Product A, 4oz/gal	0.72 ^{cdefg}	0.74 ^{bcdefg}	0.00 ^j
Product B, 4oz/gal	0.93 ^{abcd}	0.63 ^{fgh}	0.30 ^{hijk}
Product C, 4oz/gal	0.90 ^{abcde}	0.95 ^{abc}	0.50 ^{ghi}
Product D, 4oz/gal	1.00 ^{ab}	0.84 ^{abcdef}	0.66 ^{defg}
Product A, 6oz/gal	0.69 ^{cdefg}	0.65 ^{efg}	0.00 ^{jk}
Product B, 6oz/gal	0.65 ^{efg}	0.65 ^{efg}	0.00 ^j
Product C, 6oz/gal	0.74 ^{bcdefg}	0.63 ^{fgh}	0.00 ^j
Product D, 6oz/gal	0.85 ^{abcdef}	.91 ^{abcde}	0.30 ^{ik}
Control	1.08 ^a	1.02 ^a	0.93 ^{abcd}

^{a-k} Means with different superscripts differ (P < 0.05).



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To The University of Arkansas Graduate School:

Please accept this letter as an acknowledgement that Pramir Maharjan did the majority of the work described in the manuscript entitled “Evaluating different hydrogen peroxide products for residual and efficacy over time” which is part of his thesis that is entitled “Evaluation of water sanitation options for poultry production”.

Sincerely,

Susan Watkins, PhD

Professor and The Arkansas Poultry Federation Chair of Poultry Science

CONCLUSIONS

The poultry industry uses various water sources such as the municipal water, underground water, and to some extent, surface water and rain water. Regardless of the source, it is highly important that water provided should be free of microbial contamination to ensure flock health and performance, and food safety. Therefore, water sanitation is a very crucial step in a poultry operation and should be effectively carried out.

Prior to water sanitation, all water being supplied for poultry drinking purposes should be routinely tested for microbiological and physico-chemical parameters such as pH, electrolytes and minerals, organic load and microbial contamination so that the appropriate water sanitation strategies can be employed. Various brands of water sanitizers or water line cleaners are available in the market under a few classes of disinfectants advocating its efficacy under worst case conditions. These products should be monitored for their true efficacy, applicability, and cost effectiveness along with safety aspects. Water being the prime nutrient for poultry, the industry should pay close attention in these regards to providing the best sanitizing option for poultry producers and to address any type of water quality issues.